

## Penicillin sensitivity of gonococci in Ethiopia\*

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This study was made to determine whether strains of *Neisseria gonorrhoeae* with increased resistance to penicillin were present in Ethiopia. The emergence of such strains elsewhere in the world has been amply noted. This has been associated with an increase in both the number of treatment failures and the number of reported gonococcal infections. The African continent is thought to share in this phenomenon, but documentation remains fragmentary.

### Methods

Strains of *N. gonorrhoeae* were obtained from African men with acute purulent urethritis attending the Venereal Disease Clinic in Addis Ababa during the period October, 1969, to October, 1970. Because of the large clinic attendance, culture specimens were taken from the first five patients seen by one of the investigators on a single morning each week of the study period. The specimens were obtained by passage of a sterile cotton swab into the distal urethra. The swab was then placed in Stuart's transport medium (Stuart, Toshach, and Patsula, 1954) and plated within 1 hour on both chocolate agar and Thayer-Martin medium (Thayer and Martin, 1964). After incubation for 18 to 24 hrs in a 5 per cent. CO<sub>2</sub> atmosphere at 35°C, typical colonies were picked and identified by Gram stain. All Gram-negative diplococci were further identified by oxidase reaction and fermentation pattern in CTA Medium (BBL) containing 1 per cent. dextrose, maltose, or sucrose. Gonococcal strains were oxidase positive and fermented dextrose, but not maltose or sucrose. The penicillin sensitivity of the gonococcal isolates was determined with an agar dilution technique. Chocolate agar was prepared from Gonococcal Medium Base (Difco) and defibrinated sheep's blood

(5 per cent.). Serial 2-fold dilutions of crystalline potassium penicillin G were incorporated into the agar at 50°C, to give final concentrations of 0.6, 0.3, 0.15, 0.075, 0.036, 0.018, and 0.009 µg. penicillin per ml. media. The agar was poured into 100 mm. Petri dishes to a depth of 3 mm. and used on the day of preparation.

The inoculum was prepared by picking several colonies from a 24-hr growth on chocolate agar and emulsifying them in peptone water broth. The concentration of this specimen was adjusted visually to a No. 0.5 MacFarland standard. This suspension contained between 10<sup>7</sup> and 10<sup>8</sup> organisms per ml. fluid. Using a standardized loop, 0.01 ml. of this standardized suspension was inoculated on the penicillin-containing chocolate agar.

A control plate without antibiotic was similarly inoculated. A strain of *Staph. aureus* with a known mean inhibitory concentration (MIC) of 0.036 µg. penicillin per ml. was included as a control with each determination. The lowest concentration of penicillin which completely inhibited the growth of the test organism or resulted in a sudden and marked decrease in the growth of the organism was considered to be the MIC for that strain. Following the completion of this study, W.H.O. *N. gonorrhoeae* international reference strains III, V, and VII were obtained from Dr. Alice Reyn of the Statens Serum-institut in Copenhagen. The provisional reference values of these strains, expressed as the 50 per cent. inhibitory concentration (IC<sub>50</sub>) of penicillin are 0.069, 0.55, and 0.0089 µg./ml. respectively (Reyn, 1970). The MIC of each reference strain was determined ten times in our laboratory by the method described above. Their average MIC values, expressed as µg. penicillin per ml., were 0.034, 0.6, and 0.006 respectively. Thus, the MIC values of these strains determined in our laboratory correspond rather closely to their IC<sub>50</sub> provisional reference values.

### Results

The distribution of the penicillin sensitivities of 234 strains of *N. gonorrhoeae* is given in the Table. Half of the isolated strains had a MIC value to penicillin of 0.036 µg./ml. or less. We considered these strains 'sensitive'. The remaining 50 per cent. of the strains had MIC values of 0.075 µg./ml. or more and were

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considered 'resistant' to penicillin. Strains with a MIC of 0.6 µg./ml. and greater made up 16 per cent. of the total and were classified as 'highly resistant'.

TABLE Range of penicillin sensitivities of gonococci in Addis Ababa

MIC (µg./ml.)	Strains of gonococci	
	No.	Per cent.
0.0090 and less	11	4.7
0.018	48	20.3
0.036	58	24.8
0.075	45	19.2
0.150	23	9.8
0.30	12	5.1
0.60	26	11.6
Over 0.60	11	4.7
Total	234	100.2

## Discussion

The percentage of our strains demonstrating an increased resistance to penicillin would seem to be higher than that found in Europe (Lynn, Nicol, Ridley, Rimmer, Symonds, and Warren, 1970; Nielsen, 1970), but lower than that seen in Asia and western North America (Holmes, Johnson, and Floyd, 1967; Ronald, Eby, and Sherris, 1970; Reyn, 1969; Willcox, 1970; Moses, Desai, Bhosle, and Trasi, 1971). The results are similar to those reported from the Center for Disease Control in the United States (Martin, Lester, Price, and Schmale, 1970).

Limited information has been available concerning the prevalence of gonococci with increased resistance to penicillin in other African nations. In 1961, when 74 strains of *N. gonorrhoeae* from West Africa (Ghana, Chad, and the Cameroons) were tested for their sensitivity to penicillin, 99 per cent. were 'highly sensitive' (W.H.O., 1963). Clarke (1964) noted treatment results in 126 Nigerian males with gonococcal urethritis given 2.4 mega units procaine penicillin in three consecutive daily doses. Of the 98 patients who were seen in follow-up, 24 complained of continued discharge and urethral irritation. All 24 patients still harboured *N. gonorrhoeae*. Although penicillin sensitivity testing of the infecting organisms was not done, the high incidence of treatment failures suggests the presence of gonococci with decreased sensitivity to penicillin. Two recent papers have confirmed the presence of such strains in West Africa. Using a tube dilution technique, Quincke (1970) found 79 per cent. of 85 *N. gonorrhoeae* strains from Togo to be resistant to 0.05 µg./ml. penicillin. Sixty of the 85 strains were isolated from prostitutes. On the remaining 25 strains, eighteen (72 per cent.) showed resistance at this level. In

Yaounde, Millan and Huet (1970) tested eighteen strains of gonococci isolated from males with acute purulent urethritis for their sensitivity to various antimicrobial agents. On the basis of their results, using an antibiotic disc agar diffusion technique, they classified ten of the strains as 'sensitive' to penicillin and eight as of 'intermediate' sensitivity. The MIC values to penicillin were determined by a dilution method for seven of the eighteen strains. The results ranged from 0.01 to 1.0 international units/ml. and were stated to be in 'satisfactory concordance' with values obtained by the diffusion method.

Documentation of the presence of gonococci with increased resistance to penicillin in East Africa was provided by Phillips, Fernandes, Pirani, and Wagaine (1969). They found that 70 per cent. of their 193 strains were resistant to 0.06 µg./ml. penicillin or less. Arya and Phillips (1970) reported a second series of patients from Kampala correlating treatment failure with antibiotic dosage and the penicillin MIC of the infecting strain of *N. gonorrhoeae*. In this later study, 80 per cent. of 173 strains showed diminished sensitivity to penicillin.

These reports, together with our own, provide evidence that strains of *Neisseria gonorrhoeae* with increased resistance to penicillin are present in many areas of Africa. Further studies will be required before any estimate can be made of the extent and severity of the problem. The origin of these less sensitive organisms is unknown. Importation of strains from other areas is certainly possible; however, the ready availability of antibiotics throughout Africa and the widespread use of low dose repository penicillin for the treatment of gonorrhoea may well have led to the local selection of these strains (*Ethiop. med. J.*, 1970; Quincke, 1970).

## Summary

The penicillin sensitivities of 234 strains of *N. gonorrhoeae* isolated in Addis Ababa were determined; 50 per cent. of these strains were found to have minimum inhibitory concentrations of 0.075 µg./ml. of penicillin or more, and 16 per cent. required 0.6 µg./ml. or more. A review of the results of gonococcal penicillin sensitivities from other African countries is presented.

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# **Sensibilité des gonocoques à la pénicilline en Ethiopie**

## **SOMMAIRE**

On a déterminé les sensibilités à la pénicilline de 234 souches de *N. gonorrhoeae* isolées à Addis-Abéba; 50 pour cent de ces souches eurent une CMI de 0,075 µg/ml de pénicilline ou plus, et 16 pour cent exigèrent 0,6 µg/ml ou plus. On passe en revue les résultats des sensibilités du gonocoque à la pénicilline dans les autres pays d'Afrique.